Remarks/Arguments

Amendment to the Specification:

The Specification was amended to recite proper priority data for the instant application. No new matter was added and entry of the amendment is requested.

Amendments to the Claims:

Claims 1 and 2 were amended to correct typographical errors. In addition, claim 1 was amended to further define the solid support on which the polymerase is immobilized. Support for these amendments may be found on page 6 lines 10 and 26 and on Figure 3 and its description in the section entitled "Brief Description of the Drawing."

No new matter was added and entry of the amendment is requested.

Priority:

The Office Action alleged that the instant application has not complied with one or more conditions for receiving priority because the Declaration did not provide the relationship between the parent application and the instant application.

Applicants have amended the specification to recite the relationship between the instant application and the parent application (09/266,187) as requested by the Examiner. This basis of objection is now moot in view of Applicants' amendment and should be withdrawn.

Claim Objections:

The Office Action objected to claims 1 and 2 as allegedly containing a period in mid sentence. Applicants traverse because the alleged period is not present in Applicants' copy of the specification or in the published PCT (WO 00/53805). Solely in an effort to expedite prosecution, Applicants have amended claims 1 and 2 to replace the periods with commas. This objection is moot in view of the claim amendments and should be withdrawn.

35 U.S.C. 103:

Claims 1-13 stand rejected under 35 U.S.C. 103 as allegedly obvious in view of Ross (WO 91/06678) and Williams (US 6,255,083). Applicants respectfully traverse.

A Combination of Ross and Williams would not lead to Applicants' Claimed Invention

The claimed invention is directed to a method of sequencing where a plurality of polymerases are each individually attached to reaction centers on a solid support, with each reaction center located at an optically resolvable distance from other reaction centers. In the method of sequencing, a nucleic acid sample hybridized to an oligonucleotide primer is provided to the polymerase. Polymerase mediated elongation is performed with dNTPs with each dNTP being differentially-labeled with a detachable labeling group and blocked at the 3' portion. Since the dNTPs are blocked, only one nucleotide is added to the growing DNA chain and the label of this single nucleotide is detected and the DNA sequence is determined. After detection, the labeling group and blocking group is removed and a second round of polymerase mediated elongation is allow to proceed. This process is repeated and the sequence of the nucleic acid in any one reaction center is determined (See, claim 1 of the instant application).

In contrast to Applicant's claimed invention, Ross does not immobilize the polymearase on the solid support. Rather, Ross immobilizes nucleic acid templates. In addition, Ross is not a single molecule detection approach - as is the claimed invention. Ross is directed to a method of sequencing where many copies of a nucleic acid (template nucleic acid) are prepared for sequencing (Ross page 7 lines 1-2). Since Ross uses multiple nucleic acid templates, Ross' detection method is directed to detection of multiple label molecules. The detection of a label which is incorporated into a single molecule of nucleic acid, as taught in instant claim 1, is neither taught or suggested by Ross. Furthermore, the addition of Williams does not cure this defect.

Williams refers to a method of sequencing where a single molecule of nucleic acid is elongated on a solid surface. In contrast to Applicant's claimed invention (and Ross), which recites the use of blocked nucleotides, Williams refers to the detection of nucleotide addition in real time with the use of unblocked nucleotides. Williams' nucleotides comprise both a

fluorescent dye (attached to the gamma phosphate) and a quencher (not attached to the gamma phosphate) such the fluorescence of the dye is attenuated at least 5-fold. See Williams, col. 5 lines 30-54. As the nucleotide is incorporated into the growing nucleic acid chain, the polymerase cleaves and removes the gamma phosphate from the nucleotide. Since the quencher remains with the incorporated nucleotide (because it is not attached to the gamma phosphate), the fluorescent dye on the released gamma phosphate is no longer quenched and the increased fluorescence is detected. Id. Since Williams' label is attached to the gamma phosphate, it is never incorporated into the elongating nucleic acid. This is in contrast to the claimed invention which specifically requires incorporation of the detectable label (see, e.g., step (e) of claim 1). Like Ross, Williams does not teach the detection of a label which has incorporated into a single molecule of nucleic acid as in the claimed invention.

There is No Motivation To Combine the Ross and Williams

There is no motivation to combine Ross and Williams because they are directed to incompatible methods.

As discussed in the previous section, Ross is directed to a method of sequencing multiple copies of nucleic acids using blocked dNTPs (See above and also Ross, Figure 2). As each dNTP is added in Ross' method, the elongation is stopped and the identity of the dNTP is detected and recorded (Ross, page 7, lines 7-9, page 12, lines 27-29). Ross' sequencing method is not continuous because the polymerases is paused each time a blocked dNTP is added to an elongating chain. Elongation by polymerase is not started again until the added dNTP is unblocked.

In contrast to Ross, Williams is directed to "sequencing of single molecules by polymerase synthesis" (Williams, title). In Williams' method, a single nucleic acid is used as a template in the presence of polymerase and unblocked dNTPs. Significantly, "sequence information is produced continuously as polymerases continually incorporate all four nucleotides into growing nucleic acid chain" (Williams, col. 1, lines 50-56). Since Williams uses a continuous method of polymerase chain elongation, Williams does not and cannot use blocked dNTPs as required in Ross.

In summary, because Ross requires large numbers of template nucleic acids, blocked nucleotides, and detection of a population of nucleic acids while Williams requires single templates, unblocked nucleotides, and single molecule detection, a person of skill in the art would find no motivation to combine Ross and Williams.

There is no Reasonable Expectation of Success Even if the Cited References Were Combined

Even if Ross and Williams were combined, it would not lead to a functional sequencing method. Ross' and Williams' methods are incompatible. Williams is directed to DNA sequencing where "sequence information is produced continuously as polymerases continually incorporate all four nucleotides into growing nucleic acid chains" (Williams, col. 1, lines 42-49). Because of the requirement for the continual incorporation of nucleotides, Williams method cannot function with blocked nucleotides or reversibly blocked nucleotides. If a blocked nucleotide (Ross' nucleotides) is used in Williams' method, it would lead to immediate cessation of elongation and prevent the sequencing of more than one base. Further, as discussed above, Williams requires nucleotides that incorporates a fluorescent dye on the gamma phosphate and the release of this gamma phosphate bound dye, upon nucleotide incorporation, is detected and used to determine a sequence. However, Ross' nucleotides retain the label after nucleotide incorporation. Since Ross' nucleotides do not release label, it cannot be detected with Williams' method (which detects released labels) and cannot be used with Williams' method for sequencing.

In contrast to Williams, Ross' sequencing method necessitates the use of blocked nucleotides because Ross' does not detect the addition of nucleotides in real time. In Ross' method, only blocked nucleotides are used and elongation on a population of nucleic acid is terminated immediately following incorporation of the blocked nucleotide. After the blocked nucleotide is incorporated into an elongating nucleic acid, the labels from the population of nucleic acids are detected using, for example, one of the methods described by Ross (e.g., radioactive detection, mass spectrometry, fluorescence detection using non-excited spectrofluorometers as described above). Since a label is detected from a population of nucleic acids, the use of blocked nucleotides is essential. At the very least, the incorporation of more

than one labeled nucleotide at a time would prevent a determination of the order of addition of nucleotides. Since a nucleic acid sequence is determined by the nucleotide's order of addition, the sequence cannot be determined if the order is not known. Furthermore, since the gamma phosphate labels of Williams' nucleotides are released upon nucleotide incorporation, Ross' detection method, which detects the DNA chain, would not detect any signal if Williams nucleotides are used. Thus, a combination of Williams and Ross would not lead to a workable method of sequencing a nucleic acid because the Williams and Ross are incompatible. Further, such a combination, in any event, would not lead to Applicants claimed invention.

For the reasons stated above, the rejection of claims 1-13 under 35 U.S.C. §103 in view of Ross and Williams should be withdrawn.

CONCLUSION

In summary, in view of the amendments and remarks contained herein, Applicants maintain that the subject application is in condition for allowance and respectfully requests a Notice of Allowance for the application.

Applicants believe no further fee is due at this time; however, the Commissioner is authorized to charge any additional fees that may be due, or to credit any overpayment, to the undersigned's account, Deposit Account No. 50-0311, Reference Number: **18921-001 NATL** (Customer Number: **35437**).

If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

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Ivor R. Elrifi, Reg. No. 39,529
Eric Sinn, Reg. No. 40,177
Attorneys for Applicants
MINTZ, LEVIN, COHN, FERRIS
GLOVSKY AND POPEO, P.C.
Customer Number: 35437
666 Third Avenue-24th Floor
New York, New York 10017
Telephone: (212) 935-3000
Telefax: (212) 983-3115

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